Almond Variety Development

Project No.: 06-HORT5-Gradziel/Crisosto

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Interpretive Summary:

In 2006 over 6,000 seed were recovered from 16 separate crossing combinations. Approximately 95% of controlled crosses were directed towards improved yield consistency and pest/disease resistance. Approximately 40,000 seedling trees developed from previous controlled crosses between parents with promising levels of kernel quality, disease resistance and or self compatibility were evaluated. Promising selections were placed in regional evaluation plots in the Sacramento and San Joaquin Valleys. Advanced selections in previously established long-term regional trials continue to perform well, including the recently released Winters variety, the recently released FPS#1 low Bud-failure source for Carmel, the soon to be released Sweetheart variety (selection 36-52) and the promising high-yield selection 2-19E.

Effective molecular markers are being developed for self-incompatibility / self-compatibility, and key developmental processes involved in hull maturation (including hull and shell split and associated susceptibility to kernel diseases and insect pests. Molecular based markers, developed as part of a Industry-University BioStar project, allow the more accurate selection of desired traits as well as a clearer understanding of the key genetic and developmental mechanisms controlling those traits

Objectives:

Develop (1) improved pollinizers for Nonpareil, and ultimately, (2) varieties that possess self-fertility and improved disease and insect resistance. Objectives for 2006-07 include:

A. Generate the next generation of almonds from controlled crosses and screen progeny trees for self-compatibility, tree productivity, kernel quality and resistance to key pests/diseases.

B. Expand field trials of new UCD selections. Continue to monitor performance of Winters, UCD selections '36-52', '2-19E', low-BF Carmel sources, and the Nickels rootstock.

C. Continue to develop rapid selection/breeding techniques for self-compatibility, Noninfectious Bud-failure, disease resistance, and pest (especially NOW) resistance.

Results and Discussion:

A. In 2006 over 6,000 seed were recovered from 16 separate crossing combinations. Approximately 95% of controlled crosses were directed towards improved yield consistency and pest/disease resistance. Disease and pest resistance in initial seedling trees are being assessed through evaluation of natural infections in seedling blocks. The bulk of field activities in 2006-2007, however, involve the evaluation of the approximately 40,000 seedling trees developed from controlled crosses between parents with promising levels of kernel quality, disease resistance and or self compatibility. Tree and nut data are being collected/analyzed to determine the value of various parental crossing combinations, to rouge-out or eliminate ~80 % of 3rd & 4th year trees (to reduce field costs while allowing a more detailed quality assessment of remaining crossing progeny in subsequent years) and to select promising genotypes for regional testing in anticipation of future variety releases.

B. Field trials are currently underway for three groups of UCD almond selections: a) recent releases including the 'Winters' almond and the low Bud-failure Carmel source (FPS#1); b) advanced selections being considered for release, and c) UCD experimentals being evaluated for regional adaptability, disease/pest resistance and yield.

2006	kernel	Shelling	
Variety	weight (g)	%	Yield/A
Carmel	1.22	52.0	3708
Winters	0.98	52.7	3359
Nonpareil	1.17	55.9	3002

Table 1. Performance of the 'Winters' variety at the Butte Regional variety Trial in 2006.

The Winters variety continues to show consistent, high yields in several Sacramento Valley evaluation trials (Table 1, Fig. 1). Yields in the Kern County Regional Variety

Trial are lower, being comparable to the variety Carmel due to the high incidence of alternaria leaf spot disease at this site (to which both Winters and Carmel are susceptible) (Fig. 3). Field studies in 2006 have confirmed a low level of self-fertility in Winters, which, while not sufficient to produce consistently high levels of self-pollination, appear to contribute to its good cropping consistency, particularly in years with poor weather during flowering. Winters was developed as a pollinizer for the early Nonpareil bloom and its flowering period has consistently shown good bloom overlap with early Nonpareil bloom over the last 10 years of evaluation (Fig. 2).

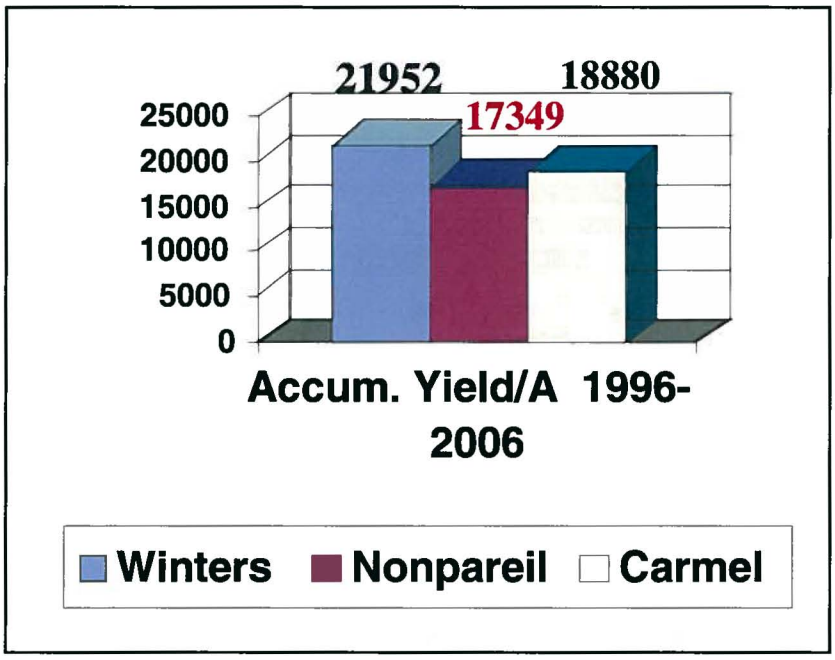


Fig. 1. Ten year yield of 'Winters' relative to 'Nonpareil' and 'Carmel' at the Butte Regional Variety Trial.

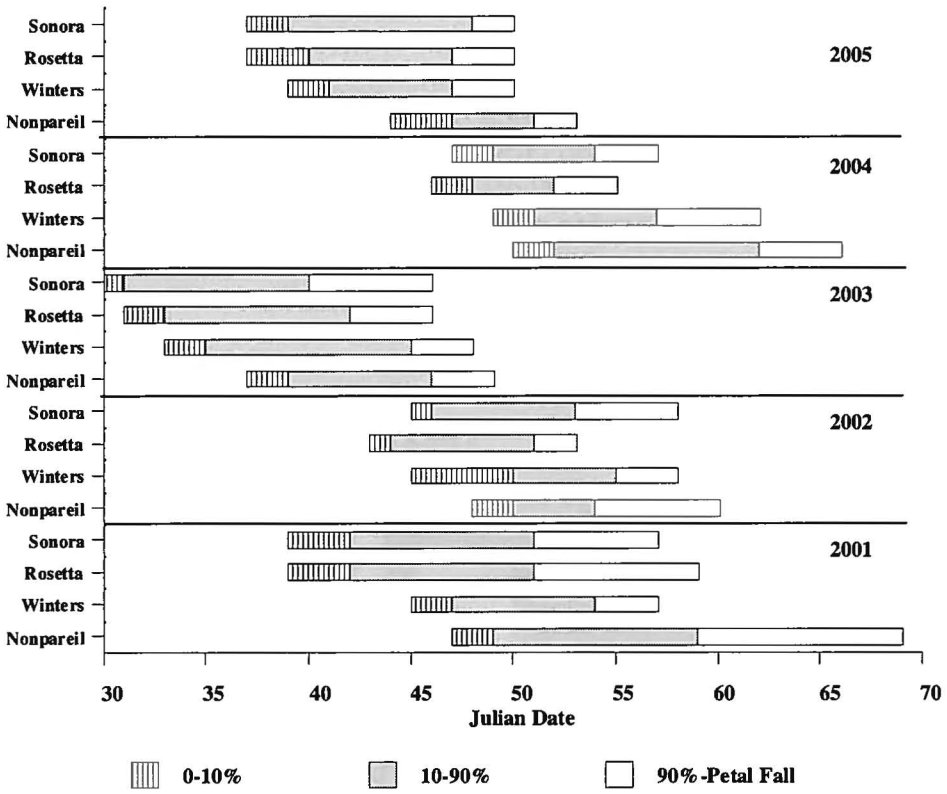


Fig. 2. Bloom progression of 'Winters' relative to 'Nonpareil' and the widely planted early flowering cultivars 'Sonora' and 'Rosetta' from 2001 to 2005 at the North Sacramento Valley evaluation site.

The low bud- failure Carmel source (FPS#1) continues to show low levels of bud-failure in the ongoing 16-year-old evaluation plot in Kern County. California nurseries have utilized this breeding selection as one of their principal propagation sources when establishing their mother-block trees, (i.e. the trees used to propagate grower stock). Because these nursery trees are one to two generation removed from the FPS #1 source they show greater variability in bud-failure expression. Major California nurseries are currently evaluating these individual tree sources, using procedures developed at UCD, to identify individual trees whose vegetative progeny result in the lowest levels of bud-failure possible.

Concurrent with the selection and testing of the more traditional advanced lines, leading to the release of Winters and low BF Carmel clones, the breeding program has emphasized the incorporation of new germplasm having improved resistance, self-compatibility and improved kernel size and quality. For example, UCD 36-52 continues to show very high kernel quality (high oleic acid which confers good processing quality and phytonutrient value and lower susceptibility to rancidity in storage), good productivity, partial self-fertility, and improved resistance to navel orangeworm and associated aflatoxin contamination. Because of its positive attributes and its potential as an alternative/replacement variety for the premium

quality, niche variety 'Marcona', UCD 36-52 is presently being prepared for release to the California industry.

UCD 2-19E is a very productive but potentially alternate bearing pollinizer for the Nonpareil late-bloom. New plantings of this selection have recently been established in Sacramento Valley and San Joaquin Valley test plots to determine whether consistent high yields are possible with appropriate irrigation/fertilizer management. Figure 3 plots the production of UCD Selection 2-19E relative to Nonpareil, Carmel and Winters at the Kern RVT. While Carmel and Winters show sizable crop loss in years (labeled A) following high Alternaria Leaf Spot damage, Selection 2-19E shows more tolerance to Alternaria but is prone to alternate bearing.

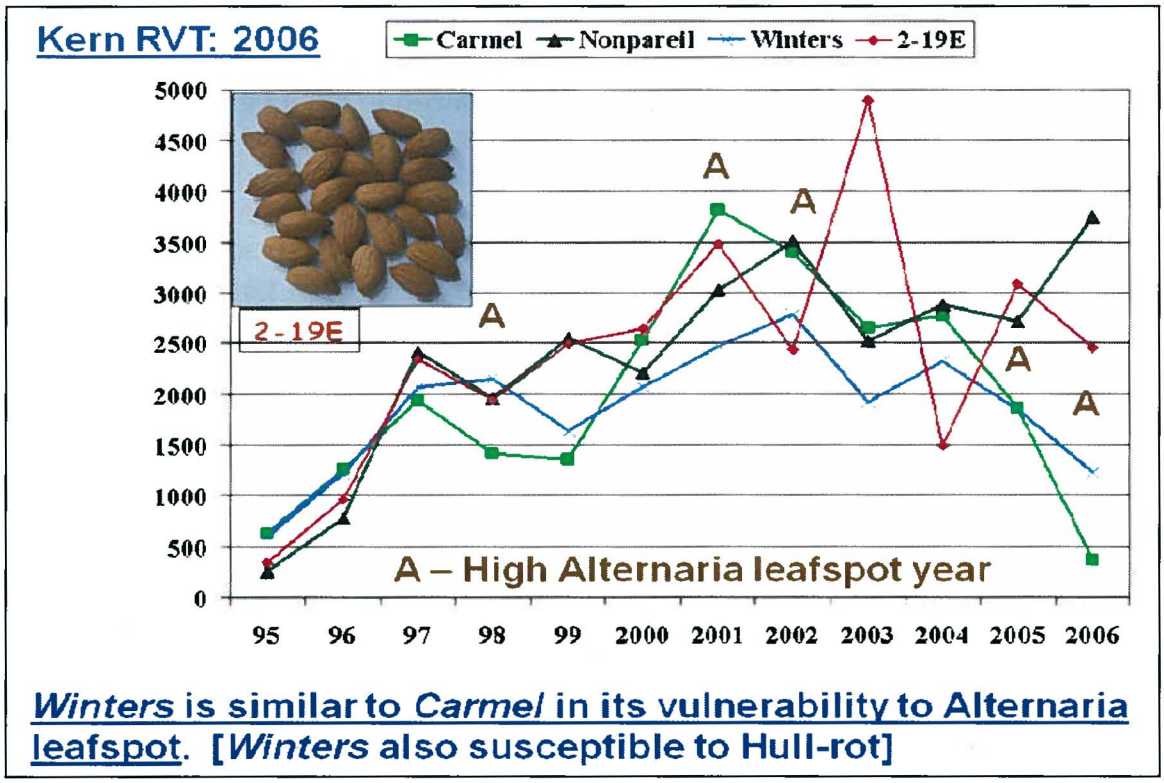


Fig. 3. Production of UCD Selection 2-19E relative to Nonpareil and Winters at the Kern RVT.

In 2004, 22 advanced UCD experimental selections, demonstrating promising levels of self-compatibility, pest/disease resistance, kernel quality and/or tree productivity, were planted in medium-scale (10-100 tree) grower test plots in Kern and Colusa Counties. Results from the first (2006) harvest have identified several individuals with particular promise but have also identified several individuals with possible deficiencies (disease susceptibility, undesirable tree structure, low yields, etc.). In 2006, 10 additional UCD experimental selections possessing improved disease/pest resistance and in some selections self-compatibility were propagated for planting in 2007 in Sacramento and San Joaquin Valley test sites having high insect/disease pressures. Tree and kernel characteristics of the 10 most promising seedling selections are presented in Appendix B.

C. Since genes ultimately determine specific plant characteristics, the best selection marker for a difficult to distinguish trait such as self-compatibility or disease resisance, is the gene itself. The recent explosion in knowledge in the area of molecular genetics has made the identification and utilization of such molecular markers feasible. Molecular markers developed with Dr. Dandekar are proving accurate and efficient in identifying California self- and cross-incompatibility groups. Continued improvements in 2006 include the determination of full-length sequences for 18 California almond S-alleles as identified in 44 California varieties (see Fig. 11). Resulting data has allowed the classification of these varieties to a comprehensive listing of cross-incompatibility groups (Appendix A). This knowledge is necessary for growers to plan orchards with the required cross-compatibility among cultivars.

   In addition, specific markers for the self-compatibility (self-fertility) sources used in the breeding program are being effectively utilized to select the most promising breeding lines for further tests/controlled crosses. Molecular markers to identify the specific self-compatibility alleles present in advanced selections also allows the formulation of controlled crosses resulting in up to100 percent self-compatible progeny. (This is achieved by selecting as pollen parent, a self-compatible selection having a self-incompatible allele in common with the seed parent. Then, only pollen containing the self-compatible allele will be compatible with and so set seed on the seed parent utilized).

   Molecular marker based strategies are now being developed to better understand and positively manipulate both bud-failure and important pest/disease problems. Towards this goal, paired, clonal propagation sources demonstrating either high or low BF potential/disease resistance have been identified. Individual sources have been propagated and are being maintained under cultural/environmental conditions to stabilize BF/resistance potential and to make them available for ongoing molecular/genetic studies. Libraries of genes from Nonpareil (BF/NOW/Aflatoxin/Plum rootstock incompatible-susceptible) and Mission (resistant) continue to be cataloged and putative function predicted from analogous but more fully characterized genes from other species (in collaboration with Drs. Dandekar and Crisosto). Resultant genetic sources and gene libraries are being used as a starting point for collaborative studies to develop molecular diagnostics for susceptibility/resistance to BF, disease, insect, and environmental injury.

BioStar initiative to maximize breeding efficiency using recent biotechnologies.

To accelerate progress in developing efficient molecular markers for improved crop quality and disease/pest resistance, the 2006 Almond Variety Development and Regional Testing programs underwent sizable expansions in research efforts though without increases in the level of industry support. In previous years we have maximized our variety development and regional testing efforts (also with very limited increases in industry funding) by combining day-to-day activities of the almond breeding program with a similarly industry-funded peach breeding program. Because the more intensive

almond and peach field management operations (planting, pollination at flowering, harvest, etc.) were largely sequential with little overlap between the two crops, we were able to maximize our labor and equipment efficiency. The greater incentive for combining these seemingly disparate breeding programs, however, involved our long-term efforts to enrich the previously very narrow genetic variability within each crop through the exchange of genes between these and related species. This more exotic germplasm also has unique value for basic research on genetic control of plant development, allowing us to secure additional outside funding to further supplement breeding efforts. Previous industry reports have demonstrated the successful transfer to almond of a wide range of useful genes from peach and its wild relatives, including resistance to Navelorange worm, aflatoxin producing Aspergillus flavus, bacterial blast, as well as genes conferring desirable effects on tree structure and crop quality (including higher kernel oil quality). Because genes are physically linked together on the DNA strand, the transfer of the small pieces of DNA containing desirable genes inevitably result in some undesirable linked genes being transferred as well. Over the last 6 years we have been involved in a rigorous, recurrent (generation-by-generation) selection for desirable genes and against undesirable traits as a way to purge out unwanted traits. This process, known as gene introgression, is relatively inefficient in tree crops because of the long generation-to-generation time periods involved, and because undesirable traits (such as high chilling requirement) may be difficult to detect in any one season due to their ambiguous expression and/or differences in expression in changing environments. The emerging biotechnologies offer powerful tools to address these problems but are very expensive. In addition, because this technology presently provides the 'cutting-edge' of our understanding of genetic/developmental processes, the majority of current biotech funded research is directed towards more basic questions such as identifying gene function (gene discovery). Rather than a barrier, we saw this emphasis of biotech research on 'gene discovery' as a potential advantage. A major (though frequently poorly appreciated) problem for biotech-based gene discovery research is that to be 'discoverable' the target needs to be a single gene whose expression is pronounced enough to be identifiable in an otherwise chaotic genetic/environment background. Because of the inbred nature of most of today's crops, the genetic variability is low and so presence of these novel genes is less likely. For example, a biotech-based program to identify a commercially useful anthracnose resistance gene in traditional California almond germplasm would be unsuccessful simply because no such gene is present in the germplasm. Molecular biologists are realizing that to increase the probability of success, they need to examine genetic populations with the often conflicting attributes of being both genetically diverse yet well characterized enough to demonstrate the presence of genes with significant commercial value. During the last 15 years, the California processing peach and almond breeding programs have successfully introgressed and partially characterized a wealth of new genetic material available for crop improvement. By pooling this resource with the demonstrated expertise in molecular and postharvest fruit analysis of Carlos Crisosto's lab at the Kearney Agricultural Center in Parlier, California, we have recently been successful in securing matching funds to current almond and peach industry support to develop molecular tools to complement our more traditional gene-discovery methods and so more efficiently exploit this rich germplasm. To encourage collaboration with the

best molecular researchers, (and to facilitate paperwork), matching funds go to the more expensive molecular research with the applied breeding efforts utilizing the industry sponsored portion. A goal of this report is to document our progress in identifying and characterizing candidate genes for more detailed molecular analysis. A second objective is to illustrate how this molecular technology can dramatically increase the efficiency and so effectiveness of the applied variety development and regional testing programs. In addition, examples of the frequently significant, though often poorly recognized, limitations of this new biotechnology will be discussed.

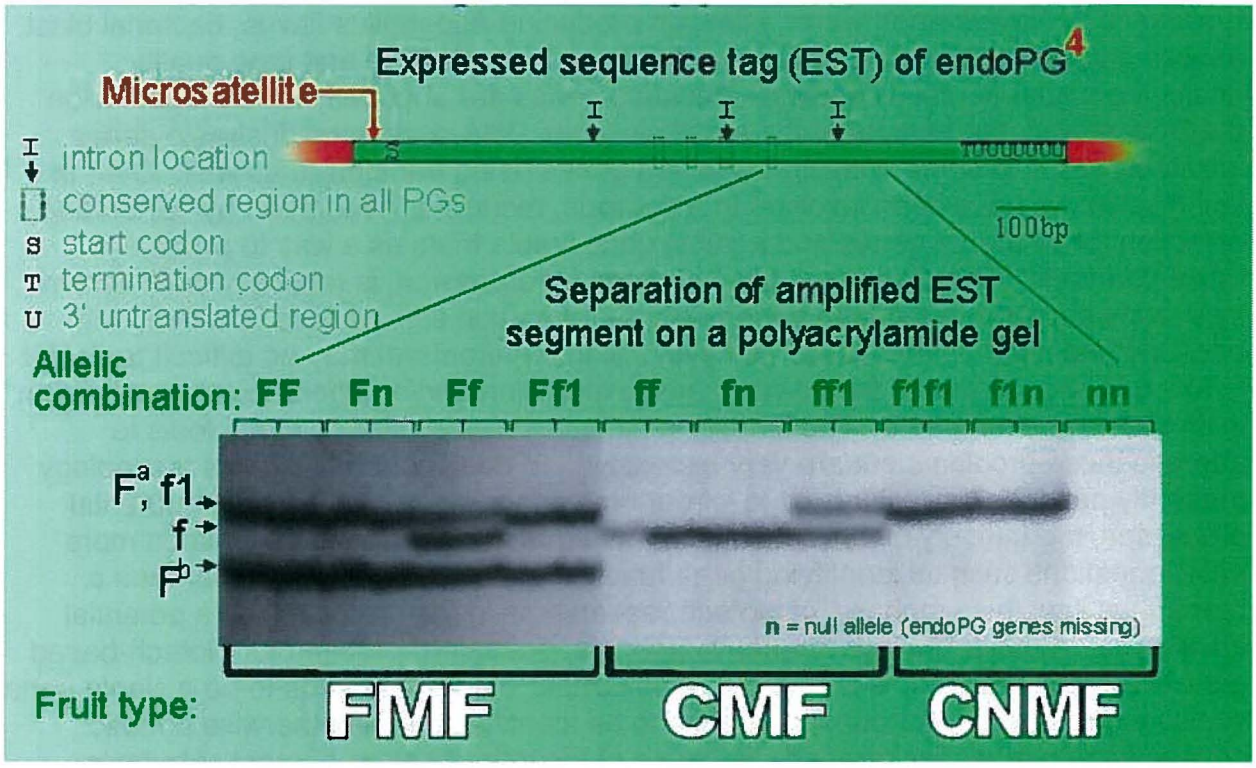


Figure 4. Gel banding patterns identifying different endoPG type genes (F & f) as well as different forms or alleles of the f-genes, and their associated fruit types.

EndoPG as a prime candidate gene controlling fruit/hull maturation. Because endopolygalacturonase (endoPG) was previously shown to be associated with softening in peach and associated with hull split in almond, the different molecular forms of this gene were studied in our almond and peach breeding lines as well as varieties, and progeny from the peach x peach and almond x peach derived crosses. Results are summarized in Fig. 4, where ESTs, (i.e. expressed sequence tags or sequences of genes being actively expressed) of endoPG at fruit/hull maturation are compared. The two distinct dark bands associated with freestone melting flesh peach types (FMF) show that 2 separate but related genes of endoPG are being expressed (F and f). In the clingstone/melting flesh (CMF) peach types only the upper band was present though for some individuals a closely paired, double-banding was apparent. The absence of the

Fig. 5. Almond (left) showing hull which analogous to the flesh of the freestone peach. The middle layer of the almond shell is composed of vascular fibers (right) which after passing through the limiting pores of the outer shell become the basis of the hull tissue (analogous to the peach flesh fibers). A clingstone-type almond is at right.

lower FMF band in all clingstone (CMF & CNMF) types demonstrated the presence of only one gene ('f/f1' in Fig. 4). Since almond and peach are both diploid organisms, (i.e. contains two forms of each gene; one from the seed parent and one from the pollen parent), the tight double banding in CMF fruit types indicates that different alleles or forms of the f-gene were inherited from each parent (f/f1). Other CMF types inherited the same f-allele from both parents (f/f or f1/f1). Significantly, in all almond and processing-type clingstone/nonmelting flesh (CNMF) varieties, the freestone or F- gene was either absent or nonfunctional leaving only the f1 allele of the f-gene and in some cases no functional or expressed genes (designated *nn* or null). The finding of this close correlation between fruit type and the form of the endoPG gene appear to confirm our hypothesis that the endoPG gene is a major player in determining fruit/hull maturation. The finding that a different form of this endoPG gene appears to be responsible for the stone adhesion trait, was an added and very exciting bonus. (Since endoPG is known to be responsible for the breakdown of the intercellular matrix or glue that holds fruit/hull cells and cell strands together, its involvement in fruit softening was intuitive. The possible control of the freestone/clingstone trait by endoPG was also anticipated as a possibility, since, as is apparent in Fig. 5, the freestone trait essentially involves a breakdown of the connections between the pit-channel strands and the pit-surface).

While less is known of almond fruit (hull) maturation, it has been documented that the commercially important process of hull split is under strict developmental control and probably controlled by endoPG-like enzymes. A wide variation in hull (and even shell – Fig. 5) splitting can be found in the native almond germplasm which is genetically controlled and so, available for manipulation for future almond variety improvement.

As a final advantage, by knowing the sequence of the endoPG-genes, we were able to develop PCR-based molecular fingerprinting techniques which allowed the rapid and high-volume characterization of breeding lines and varieties.

Rapid PCR-based molecular surveys of breeding lines and varieties. Using the very rapid and efficient PCR approach for molecular (DNA) fingerprinting we were able to

evaluate over 100 varieties and breeding lines from the almond and peach breeding programs. Since over 70 of these items were commercially important varieties, we believe the results (summarized in Figs. 6 & 12) represent an accurate general picture of the genetic control of fruit softening in current varieties. Results from the analysis of all commercial varieties were consistent with our hypothesis that the endoPG gene had duplicated itself early in the evolution of almond and peach in that one form had evolved to control stone adhesion while the other form evolved to control fruit (especially suture) softening at maturation. Because this type of duplication typically occurs as a copying error when the DNA is transcribed from mother to daughter cells, they are physically linked together. This close linkage explains why freestone/melting and clingstone/nonmelting are always inherited together since the probability of a random DNA break/rearrangement to separate freestone from melting, for example, is highly unlikely within this very short DNA distance. While both genes were found to have multiple, often subtly different, forms or alleles, whenever any F-allele was present, the fruit was freestone and melting flesh. Clingstone fruit were only present when the F-allele was completely absent or nonfunctional allowing the 'f'-allele, which further determined flesh melting characteristics, to be expressed. Different 'f'-alleles determined different fruit types; if f-alleles are present, then the clingstone flesh is melting. If all alleles are f1-alleles or null-alleles (absence of functional gene) then the flesh is clingstone/nonmelting. This advanced working hypothesis is also consistent with previous genetic studies were freestone/melting (F/{any 'f'-form}) is dominant to (i.e. masks any expression of) clingstone/melting and clingstone/nonmelting. Consequently, the freestone/melting trait is rapidly purged out of any processing peach breeding program because 1) it is an undesirable commercial type and, 2) it hides or masks the expression of the commercially important 'f'-allele. Similarly, because the f-allele, (which confers clingstone/melting and is commercially undesirable), masks the expression of the commercially important f1-allele, it is rapidly purged from processing peach breeding programs.

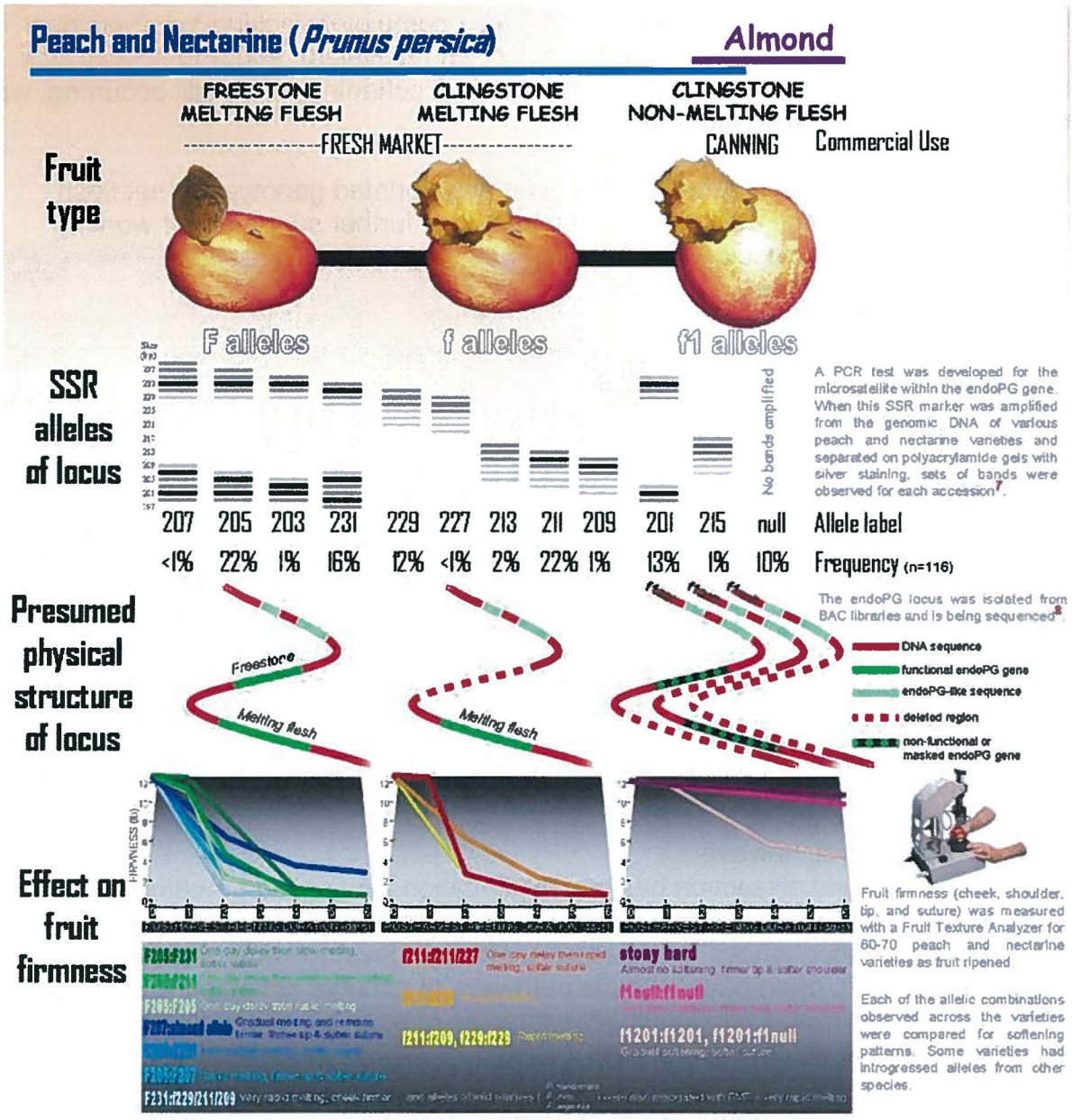


Figure 6. Sumary of fruit types, firmness & molecular data supporting the working hypothesis of 2 separate multi-allelic genes controlling pit adhesion and fruit softening in California varieties.

EndoPG expression and activity. We are presently comparing the levels of endoPG expression and activity in the different tissues of fruit types studied and the initial results support our working hypothesis and, in addition, suggest a strategy for developing improved processing peach and possibly almond types. Freestone/melting types consistently show very high endoPG gene expression and biochemical activity in both the inner and outer flesh. In nonmelting/clingstone types endoPG expression and

activity are 50-fold lower and located predominately in the inner flesh adjacent to the pit. Interestingly, in nonmelting/clingstone genotypes completely lacking any functional alleles of the F-gene and the f-gene (i.e. null or an *nn* alleles), endoPG activity was almost completely absent and post-maturation fruit softening, while still occurring, was at the lowest level of any type measured.

A high level of agreement between molecular fingerprinted genotypes, fruit flesh endoPG expression and activity, and final fruit type, further supports our working hypothesis. These molecular tools for fingerprinting (i.e. identifying specific forms of specific genes) provides the breeding program with both precise knowledge of the genetics involved as well as a more integrated knowledge of how these genes interact to determine different fruit types. Thus, these molecular approaches offer powerful tools to dissect or separate individual mechanisms conferring fruit firmness and may allow a more efficient recombination of the most desirable genes to achieve commercial goals. While a powerful tool, however, this type of molecular dissection has certain limitations in applied breeding programs because, on a whole-plant level, individual genes frequently have multiple (and often unanticipated) consequences, and because different developmental processes such as fruit/hull maturation, often have multiple pathways to achieve developmental goals. The final section of this report will document examples of such situations to highlight their potential barriers to achieving breeding goals.

Figure 7. Fruit of Fla9,20-C showing the intense red-stained pit associated with the null condition for the endoPG gene.

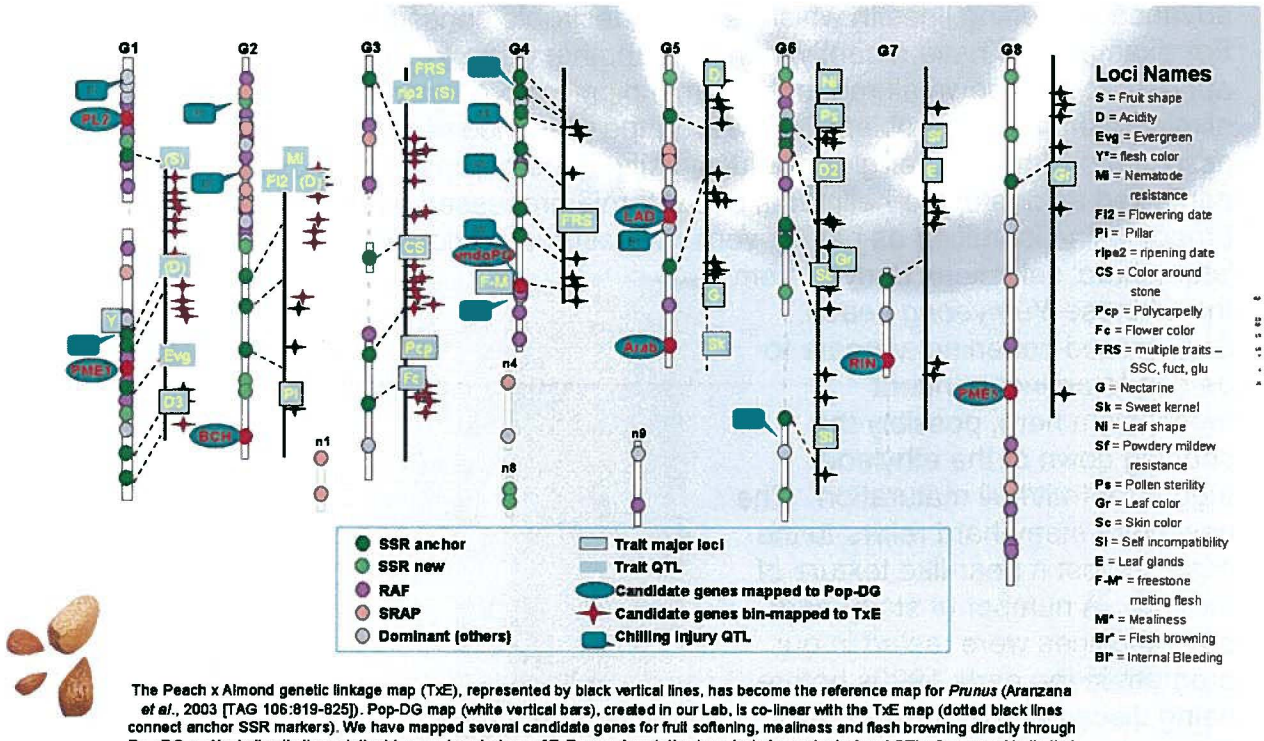
Dangers of over-dependence on molecular-based strategies for crop breeding. Based solely on our molecular findings, the development of null endoPG fruit types (i.e. complete absence of fruit flesh endoPG gene expression and biochemical activity) would be a highly desirable goal for processing nonmelting/clingstone peach breeding as it is associated with the lowest rate of post-maturation flesh softening. In fact, for the past 10 years our breeding program has been using the parent Fla9, 20-C (a very low-chill clingstone/nonmelting fresh market peach developed at the University of Florida from Mexican germplasm), which has been identified as a double null. [It was the early analysis of this breeding selection which first identified the occurrence of null alleles for this gene]. Fig. 7 shows fruit samples of this selection. While conferring desirable fruit firmness in progeny from controlled crosses, the trait is also always associated with an intense red pigmentation in the pit cavity in both the parent as well as breeding progeny.

We are presently using the molecular tools developed to target null genotypes in advanced breeding lines in which these undesirable consequences might be minimized. For example, we have recently identified another gene (designated *high-liter*) which appears to shut down all red anthocyanin pigment production in the flesh and so could eliminate the red-pit problem. (However, the presence of *high-liter*, itself, appears to be associated with a poorer gloss or luminosity of the processed fruit, which is presently perceived as one of the hallmarks of California processed peach quality). Another breeding line identified as having very low post-maturation fruit softening rates is the 'stony-hard' selections derived from the Chinese *Yumyeong* peach. Suppressed softening appears to be due to an independent mechanism here, possibly the shutting down of the ethylene trigger for fruit/hull maturation. The descriptor stony-hard refers to the crisp, almost a pear-like texture of the fruit. A number of stony-hard breeding lines were tested in our program in the early 1990s before being discarded because of the associated undesirable decline in processed fruit textural qualities (fruit were firm but 'woody' in texture).

Figure 8. Extra-Late #5: a fruit type that maintains high fruit firmness independent of the endoPG gene.

While the molecular identification/dissection of endoPG-based flesh softening has identified promising endoPG combinations and provided the tools to generate/monitor their precise recombination, this molecular approach has also proved important in identifying fruit firmness pathways independent and so possibly complementary to the endoPG activity. (Though it was only with the recent application of the endoPG molecular analysis that we became aware of the independence and so opportunities of these alternative pathways). An example is seen in the advanced 'long-keeper' Extra-Late processing peach breeding selections (Extra-Late#4 to#7) presently being placed on regional grower evaluations. Although possessing the f1-allele, associated with typical clingstone/nonmelting processing peaches, these selections appear to suppress endoPG softening of ripe fruit to levels comparable to the double null and stony-hard genotypes, yet without the undesirable characteristics (Figs. 6 & 8). (Because of the recent almond-origin of these lines, however, opportunities for undesirable linkages are relatively high, requiring thorough testing at both the field and molecular level before consideration for varietal release.

Fig. 9.



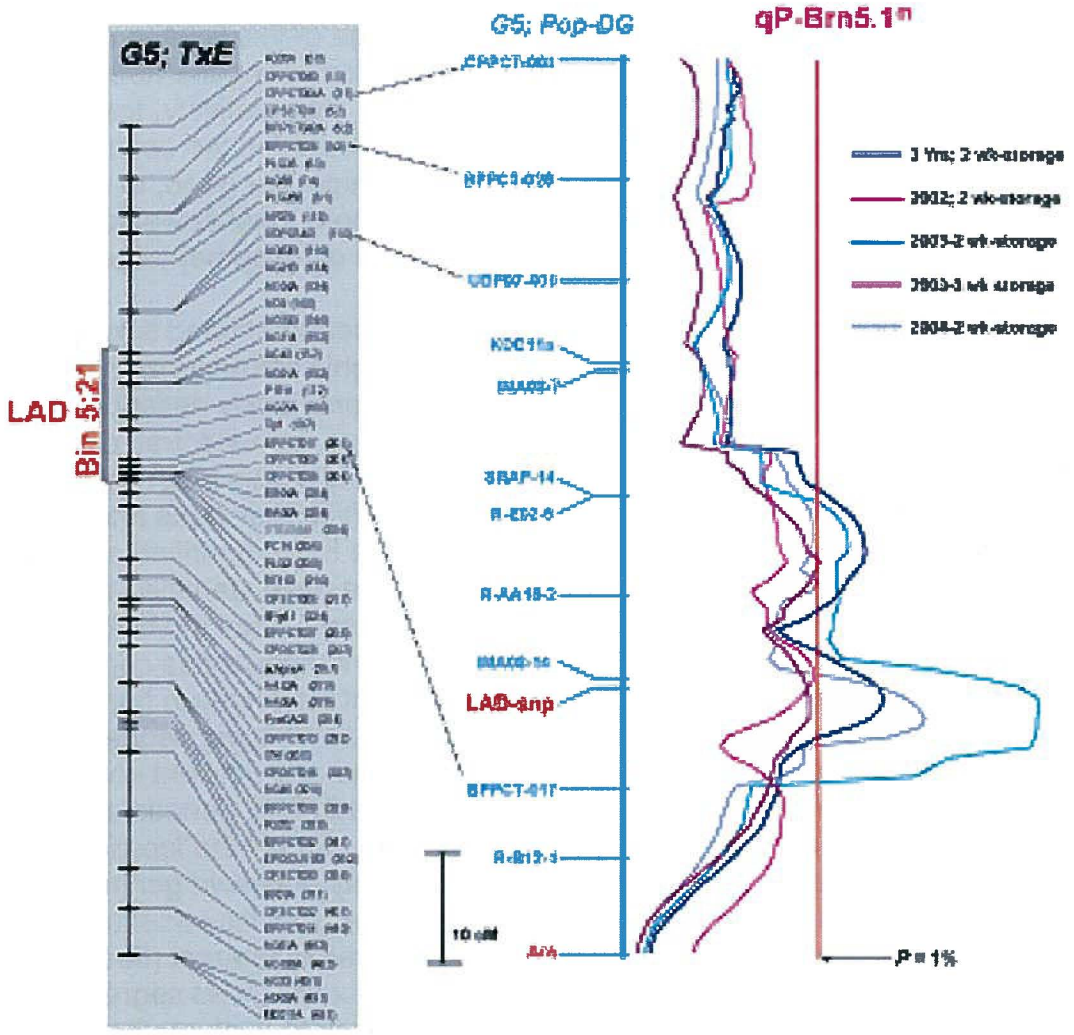
The Peach x Almond genetic linkage map (TxE), represented by black vertical lines, has become the reference map for *Prunus* (Aranzana *et al.*, 2003 [TAG 106:819-825]). Pop-DG map (white vertical bars), created in our Lab, is co-linear with the TxE map (dotted black lines connect anchor SSR markers). We have mapped several candidate genes for fruit softening, mealiness and flesh browning directly through Pop-DG and/or indirectly through the bin mapping strategy of TxE map. Annotation here includes major loci and QTLs for several traits that have been mapped in *Prunus* (those mapped in our Lab are asterisked in the legend)

Future research. Fruit /hull maturation via the endoPG and complementary pathways, while a major objective of our collaborative efforts with Carlos Crisosto's molecular group represents only one of the research avenues being evaluated within the larger crop quality context. With Cameron Peace and Ebenezer Ogundiwin, the molecular biologists directing the molecular testing, we are targeting a large number of traits of commercial importance to almond and peach. In addition, we have leveraged this research momentum to enlist the cooperation of a large number of molecular biologists doing similar research at other national and international programs. The combined efforts of these basic and applied researchers have resulted in the development of an ambitious 'roadmap' or plan for a more aggressive application of molecular tools to achieve applied breeding goals. Central to this progress has been the development of a relatively complete genetic map of almond and peach (Fig. 9) by combining the research results from these national and international programs. The resultant genetic linkage map shows the location of a large number of different molecular markers (i.e. the specific DNA sequence as determined by different molecular approaches, PCR, SSR, etc.) as well as the location of known genes controlling important traits. The map can be used to evaluate the presence of desirable and/or undesirable linkages for candidate traits. For example in Fig. 9, the stick-image labeled 'G 4' represents chromosome #4. Towards the bottom of the image, we can see the location of endoPG gene determined from our research. The location or locus for the 'Bi' gene controlling internal flesh bleeding is very close to endoPG indicating that in certain selections (as a possible example, the red-stained double null selection Fla9,20-C described above) specific desirable forms of the endoPG allele (the null form in this case) may be

physically linked and so almost always inherited with an undesirable forms of the Bi gene (conferring red anthocyanin bleeding in the pit area). If this association were confirmed, breeding programs could target the breakage of this linkage using molecular markers to select at even the seedling stage (and so eliminating the need to grow the very large numbers of plants required to field maturity). Alternatively, the breeder could target the CS (color around stone) gene known to be located in the central portion of chromosome 3 (G3 in Fig. 9) or the yet to be mapped high-liter gene, to suppress all red anthocyanin production in plants whose other genetic components would encourage it (i.e. shut down anthocyanin production upstream in the developmental pathway).

Finally, the high number of molecular markers on this peach-almond linkage map provides effective markers to identify and more thoroughly characterized additional traits of commercial value. For example, to characterize the previously described *high-liter* gene, we are mapping both molecular markers as well as *high-liter* trait expression in progeny from controlled crosses. Due to the high density of this map, it is likely that certain molecular marker are located very close to the *high-liter* locus and so will almost always segregate with it in breeding progeny. Once we have identified a molecular marker which co-segregates with the trait of interest, we have identified the general location of that trait as well. Once identified, the entire sequence of DNA within that region can be determined and individual embedded gene sequences compared against known sequences of similar functioning genes. (Recent advances in genomics and informatics have made accessible huge databases of known gene sequences and function, as well as computer software allowing the rapid and efficient comparison with hundreds of thousands of established sequences.) In addition, genetic and molecular mapping at the very high resolutions now possible, can identify the location (and through subsequent sequencing, the identity) of currently unknown genes. An example is seen in Fig. 10 where the level of association between specific molecular markers (here on a small segment of chromosome 5), and the trait of interest (flesh browning) was compared over multiple years in our *Georgia Belle x Dr. Davis* segregating peach population. The level of flesh-browning is normally difficult to evaluate because it can vary greatly depending on environmental factors such as temperature and tissue stress. A multiyear, molecular mapping of this trait, however has identified a gene locus which is estimated to account for approximately 40% of all flesh browning observed in this population. In addition to identifying the presence of this gene, this approach has also identified the specific location (the qP-Brn5.1 region on chromosome 5) of the gene which, in turn, allows its sequence determination and ultimate identification using procedures described above. The sequencing of the flesh-browning locus will also allow the development of very precise molecular probes which can identify/utilize other forms of this gene which may be inactive (no flesh browning) for our breeding population (assuming such a gene exists). The sequence will also allow the development of transgenes which might effectively turn off this gene in future genetically engineered varieties.

Fig. 10.



Pop-DG peach linkage map is co-linear with the TxE *Prunus* reference map (Dirlewanger et al.,2004). Four SSR markers anchored linkage group 5 (G5) of Pop-DG to G5 of TxE (dotted lines). LAD was bin-mapped to bin 5:21 corresponding to qP-Brn5.1m, a major QTL explaining ~40% of variation observed for flesh browning. qP-Brn5.1m was significant in each of the three years (2002-2004) and two 5ºC storage regimes (2 & 3 weeks). SNP polymorphism enabled direct linkage mapping of LAD (LADsnp) to the qP-Brn5.1m region on G5 of Pop-DG.

An example of the power of DNA sequencing in almond is demonstrated in Figure 11, where the sequence for several of the flower (pistil) functioning SI (self-incompatibility) alleles were determined in collaboration with Dr. Dandekar. By examining sequence differences between the different SI allelic forms, we can not only accurately distinguish different types but also gain understanding on the function and evolution of this important gene. In addition, by sequencing the DNA adjacent to this locus we have identified the putative gene controlling pollen recognition of self-incompatibility.

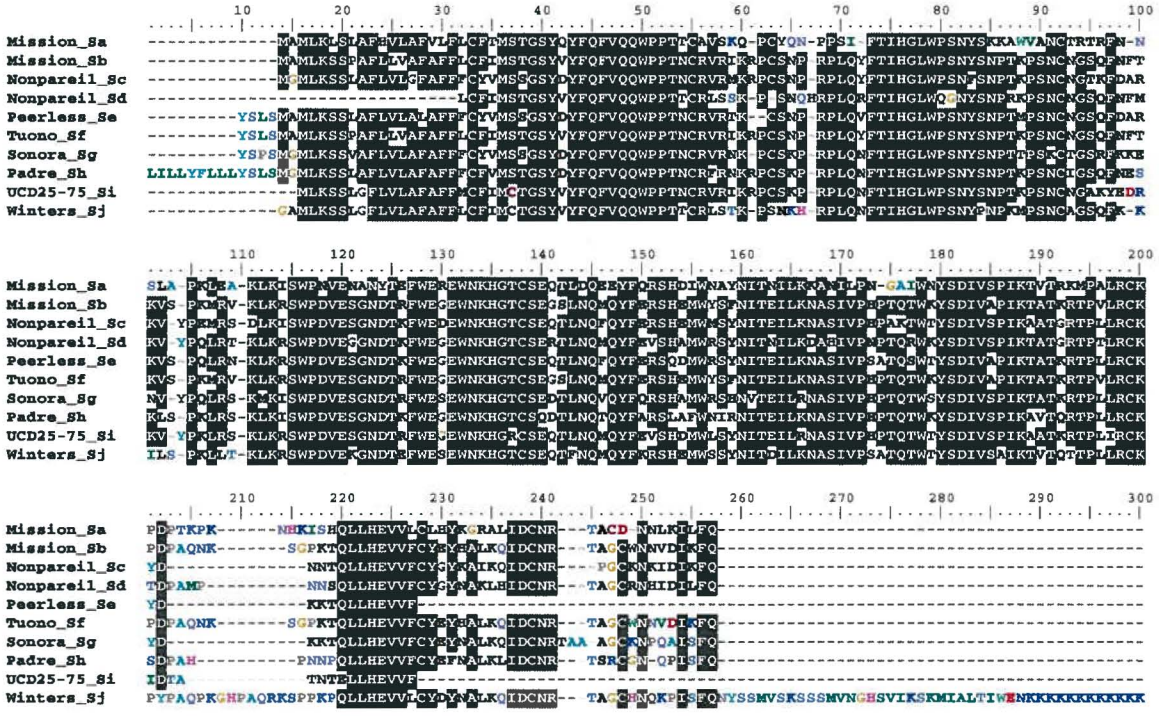


Fig. 11. Multiple amino acid alignment of S-RNases. Conserved regions boxed and labeled C1-C5. Homology between alleles shown by shading. Two conserved Histidine residues occur in regions C2 and C3.

Using the huge genomic and informatics databases developed in ongoing national and international molecular research, the structure and putative function of the gene in question can often be predicted. (It now appears to be an 'F-box' class of gene which have been shown to be important in development in other plant species).

Increasingly, this 'forward-looking' approach to determining gene function from DNA sequences can also be used in the reverse direction. For example, if the ongoing research by Rick Bostock's lab identifies a specific phenolic compound associated with Monilinia (fruit & hull rots) resistance, we might be able to predict a rough sequence for the controlling gene by examining gene sequences for that or similar phenolics from other plants (Arabidopsis, corn, tobacco, etc.) that are presently is listed in the genomic database. We could then use that rough sequence to develop molecular probes to specifically tag and efficiently identify the desired gene form even in seedling populations or in environments not conducive to disease screening.

In summary, results from only the first year of research have shown impressive increases in our genetic knowledge and so breeding potential. However, for proper application this knowledge needs to be tempered with field-based experience in the traits of interest, particularly concerning potentially associated undesirable effects. A

second danger from the unbalanced use of molecular approaches to pursue breeding goals is the risk of too narrowly focusing breeding efforts (for example, targeting the null form of endoPG as the sole strategy to control fruit softening). Not only does such a narrow perspective increase the risk of 'dead-end' research if unacceptable negative consequences are eventually recognized, but it eliminates the opportunities to identify independent genetic solutions which may avoid undesirable associations. This is particularly true in almond breeding given the huge amount of genetic variability (and so opportunity for novel trait breeding) when compared to peach and the other Prunus crops. This variability is clearly apparent when characterizing the different forms or alleles of endoPG (Fig. 12) where almond and its close relatives have demonstrated a new allelic form for almost every new accession analyzed compared with the considerably reduced genetic variability observed for the other Prunus crops.

We believe this project has achieved its goals of dramatically increasing molecular tools available to applied almond breeding (at no additional cost to industry sponsors) while concurrently allowing significant advances in our understanding of basic development pathways. This was, in large part, achieved by exploiting the inherent genetic variability developed by applied breeding programs in their (typically) multifaceted approaches to targeted breeding goals. By fully exploiting their individual strengths, both applied and basic progress is accelerated.

Fig. 12.

Other *Prunus* species and crops

Prunus species can be intercrossed, enabling allele introgression into any crop!

Close peach relatives (*P. davidiana, P. ferganensis, P. mira, P. kansuensis*)

Species included: 4
Accessions surveyed: 15
Alleles observed: 8

Two gene copies were detected in some accessions

Almond and relatives (*P. dulcis, P. argentea, P. scoparia, P. webbii,* and several others)

Species included: 11
Accessions surveyed: 40
Alleles observed: 39

Two gene copies were detected in some accessions

Apricot, Plum, and relatives (*P. armeniaca, P. domestica, P. salicina,* and others)

Species included: 23
Accessions surveyed: 419
Alleles observed: 123

Sweet and tart cherry (*P. avium, P. cerasus*)

Species included: 2
Accessions surveyed: 26
Alleles observed: 6

Sweet cherry is the only *Prunus* species for which endoPG allelic variation has yet to be observed (20 cultivars surveyed)

Recent Publications:

Martínez-Gómez, P., Sánchez-Pérez, R., Rubio, M., Gradziel, T. M.,Sozi, G.O. 2005. Application of Recent Biotechnologies to PrunusTree Crop Genetic Improvement. Ciencia Investigacion Agraria. 32 (2).

Martinez-Gomez,-P; Sanchez-Perez,-R; Vaknin,-Y; Dicenta,-F; Gradziel,-TM. Improved technique for counting chromosomes in almond. Scientia-horticulturae. 2005 May 30; 105(1): 139-143.

Peace,-CP; Ahmad,-R; Gradziel,-TM; Dandekar,-AM; Crisosto,-CH. The use of molecular genetics to improve peach and nectarine post-storage quality. Acta-horticulturae. 2005 June, no 682(1); 403-409.

Martínez-Gómez, P., Sánchez-Pérez, F., Dicenta, W. Howard, P. arus, T.M., Gradziel.. (2006). Almond. In Chittra Kole: Genome Series Vol. 9. Science Publishers Ltd. Helsinki.

Peace, C.P., Crisosto, C.H. and Gradziel T.M. 2006. Endopolygalacturonase: a candidate gene for Freestone and Melting flesh in peach. Mol. Breeding 16:21-31.

Barckley, K.K.,S.L. Uratsu, T.M. Gradziel and A.M. Dandekar (2006) Multidimensional analysis of S-alleles from cross-incompatible groups of California almond cultivars. J Amer Soc Hort Sci 131:632-636.

Peace,-CP; Crisosto,-CH; Garner,-DT; Dandekar,-AM; Gradziel,-TM; Bliss,-FA. :Genetic control of internal breakdown in peach. Acta-horticulturae. 2006 July, no 713; 489-496.

Gradziel, T., B. Lampinen J. Connell and M. Viveros. (In Press). 'Winters' Almond: an Early-Blooming, Productive and High Quality Pollenizer for 'Nonpareil'. HortScience.

Appendix A
Cross-incompatibility chart listing California almond varieties and their designated S-allele genotype

CIG	Genotype	Almond Cultivars	European Genotype
I	S^cS^d	**Nonpareil, Tardy Nonpareil**, Grace, West Steyn, UCD F8:7-180, Galaxy	S^7S^8
II	S^aS^b	**Mission**	S^5S^1
III	S^aS^c	**Thompson, Sauret no.2, Mono, Wood Colony**, Durango, Le Grand, Wassum	S^5S^7
IV	S^bS^c	**Merced, Ne Plus Ultra, Rosetta, Price cluster**, Aldrich, Pearl, jenette, Sano	S^1S^7
V	S^aS^d	**Carmel, Sauret no. 1, Livingston**	S^5S^8
VI	S^bS^d	**Monterey**, Butte, Dottie Won, Plateau, Avalon, UCD D3-25, UCD F8:7-179, Folsom, Blue Gum	S^1S^8
VII	S^aS^i	Arbuckle	$S^5S^?$
VIII	S^bS^e	Fritz, Ruby, Peerless	S^1S^6
IX	S^bS^h	Padre	S^1S^{18}
X	S^bS^j	UCD 13-1 (Winters), UCD 36-52 (Sweetheart)	S^1S^{14}
XI	S^cS^e	Tokyo	S^7S^6
XII	S^cS^g	Milo	S^7S^{13}
XIII	S^cS^j	Jordanolo	S^7S^{14}
XIV	S^dS^e	Kochi, UCD F8:8-160	S^8S^6
XV	S^dS^g	Solano, Sonora, Vesta, Kapareil, UCD F8:7-161	S^8S^{13}
XVI	S^dS^i	UCD 25-75	$S^8S^?$
XVII	S^eS^j	Harriet	S^6S^{14}
XVIII	S^aS^j	Carrion	S^5S^{14}
XIX	S^d	Jeffries, UCD 3-6, Johlyn	S^8
XX	S^f	Tuono	S^f
XXI	S^k	UCD 54P455 (peach)	$S^?$

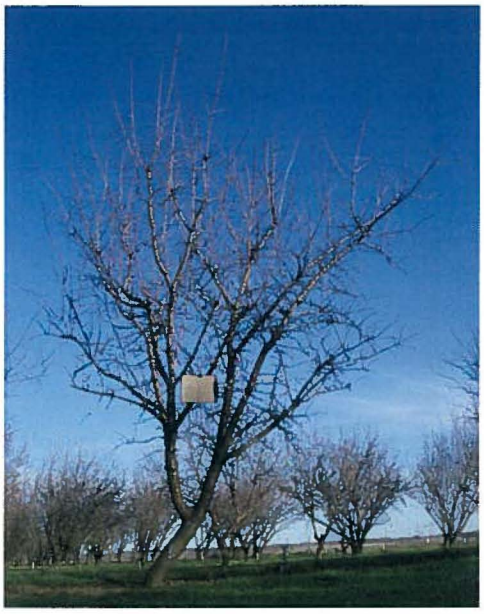
Appendix B
Advanced UCD Self-compatible Almond Selections in Regional Evaluations

Selections are self-compatible and should be cross-compatible with each other as well as all current California varieties. Honeybee pollinators will still be needed to achieve maximum yields. Because of the anticipated greater pollination efficiency of the honeybees, however, the number of hives per acre could be reduced. These breeding selections represent a very wide genetic variability due to their interspecific origins. In addition to self-compatibility, genetic options for disease and insect resistance have been observed in this material. By establishing evaluation plots for these selections in different areas of the Sacramento and San Joaquin valleys, we hope to more thoroughly evaluate the value for further resistance breeding, as well as their potential and deficiencies as possible cultivar releases. [Images of seedling trees are provided to provide information on tree growth habit. Seedling trees have not been trained or pruned and in some cases were harvested heavily for graftwood/budwood and so do not represent potential commercial form].

00A,2-3

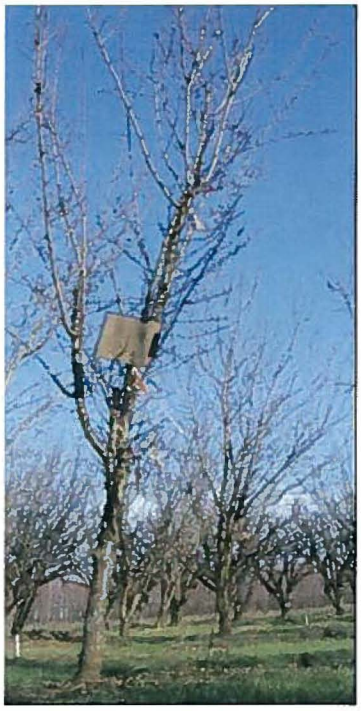
The tree is semi-upright with radial branching . Anticipated size will be 10% narrower Nonpareil but similar height. Expected bloom is approximately 7 d after Nonpareil with harvest approx. 28d after Nonpareil. Kernel quality is good however this selection may produce double-kernels. Average kernel length/width/thickness is 2.4/1.2/0.9 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crackout is 0.55. Shell-seal is also good with approximately 90% of the nuts showing complete seals. This selection resulted from a complex series of crosses involving Prunus persica (peach) and Prunus webbii in its lineage.

00A.8-27



The tree is upright-spreading and approx. 20% smaller than Nonpareil. The bearing-habit is similar in terms of the ratio of spur to shoot flower buds. The selection blooms approximately 5 d after Nonpareil and harvest approx. 18 d after Nonpareil. Average kernel length/width/thickness is 2.2/1.2/0.9 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crackout is 0.64. The paper shells give good crack out but have poor seals though the worm infestation has not been a problem to date. Kernels show good-quality though double kernels may be a problem. This selection resulted from a complex series of crosses involving Prunus persica (peach) and Prunus webbii in its lineage.

99A, 1-121



The tree is very upright, and approx. 20% smaller than Nonpareil. [*Distorted tree growth is from graft-wood harvesting.]* Bloom occurs approximately 6 d after Nonpareil, while harvest occurs approx. 28d after Nonpareil. Kernels show good-quality but double-kernels may be a problem. Average kernel length/width/thickness is 2.4/1.4/0.9 cm. Ave. kernel weight is 1.3g; kernel/kernel + shell crackout is 0.30. The shell is similar to the variety Mission, having a very good shell-seal and so low worm damage. The wild almond species, Prunus webbii as well as peach are in the lineage.

97A,1-227

Tree size is 10% smaller than Nonpareil with an upright structure. Blooms approx. 5 d after Nonpareil and harvest approx. 21 d after Nonpareil. Average kernel length/width/thickness is 2.6/1.5/0.8 cm. Ave. kernel weight is 1.5 g; kernel/kernel + shell crackout is 0.42. Shells are easily cracked yet have good seals. This selection resulted from a complex series of crosses involving Prunus mira (a wild peach species) in its lineage.

97A,1-227 at left; 97A1-232 at right. Distorted growth is from graft-wood harvesting.]

227 97A1-232

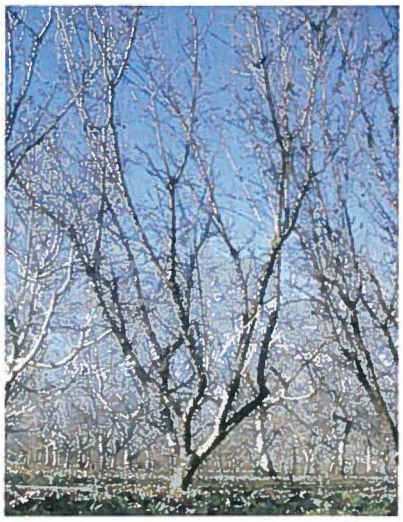
A sister line to 97A,1-227, 97A1-232 produces a more spreading tree approx. 20% smaller Nonpareil. The tree blooms approximately 5 d after Nonpareil and harvests approx. 21 d after Nonpareil. The kernels are less symmetrical than 97A,1-227, and doubled -kernels and kernel crease may be a problem. Average kernel length/width/thickness is 2.3/1.4/0.8 cm. Ave. kernel weight is 1.2 g; kernel/kernel +

shell crackout is 0.40. Shells are moderately sealed. As with 97A,1-227, the wild peach relative, Prunus mira, is in its lineage.

F7,1-1

This selection is vigorous having an upright to upright – spreading tree which can be similar to 10% larger than Nonpareil. Bloom occurs 7 to 10 d after Nonpareil. Flower densities and levels of self compatibility are high resulting in a high yield potential. Harvest occurs approximately 28 days after Nonpareil. Nuts are small and teardrop-shaped which appear desirable to the confectionery industry. Average kernel length/width/thickness is 1.8/1.1/0.8 cm. Ave. kernel weight is 0.8 g; kernel/kernel + shell crackout is 0.68. Shells are only moderately sealed. Peach (Prunus persica) was used as the source of self-compatibility found in this selection. May be bacterial blast resistant.

F8,8-160



This selection is upright-spreading resulting in a tree size similar to Plateau or Carmel. Bloom occurs approximately 7 d after Nonpareil and can be profuse. Harvest occurs approximately 6 weeks after Nonpareil. Average kernel length/width/thickness is 2.2/1.2/0.9 cm. Ave. kernel weight is 1.0 g; kernel/kernel + shell crackout is 0.57. Shells are medium in thickness and are very well sealed giving good protection against

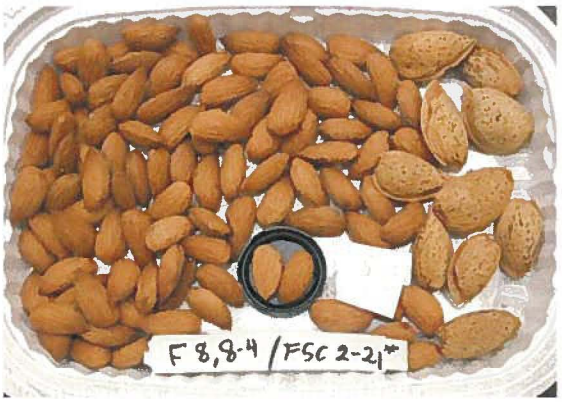
worm damage. Kernels are medium in size and of good quality. For this selection, Prunus fenzliana, a wild almond species, was used as a source of self-compatibility as well as resistance.

F8,8-161

This selection is a sister line to F8,1-160 and the tree is upright and similar in size and vigor to Fritz. Bloom occurs approximately 10 d after Nonpareil and is also profuse. Harvest occurs approximately 28 d after Nonpareil. Shells are medium and thickness and are moderately well sealed. Kernels are medium in size and of good quality. Average kernel length/width/thickness is 2.3/1.2/0.8 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crackout is 0.63. Doubles may be a problem and Alternaria leafspot lesions have been observed in early San Joaquin valley plantings.

Prunus fenzliana, a wild almond species, was used as a source of self-compatibility.

F8,8-4



The tree is upright and approx. 10-20% smaller than Nonpareil. Flowering occurs 1-2 d after Nonpareil. Flowering has been profuse (high flower density) making it a good pollinizer for the later Nonpareil bloom. Harvest occurs approx. 30 d after Nonpareil. Average kernel length/width/thickness is 2.1/1.2/1.0 cm. Ave. kernel weight

is 1.1 g; kernel/kernel + shell crackout is 0.55. Shell seal is moderately good (approx. 60% sealed). Kernels have good size, shape and texture. (Sample Nonpareil and Carmel kernels shown inside grey circle). Double kernels may be a problem under high production. This selection resulted from a complex series of crosses involving Prunus persica (peach) and Prunus webbii in its lineage.

F10D,1+2-25 Amaretto-flavored panning almond (self-incompatible)

F10D,1+2-25 is being tested as a **specialty-market, panning almond**. It is **not self-compatible and has not been noted as having promising disease resistance.** It produces a more spreading tree approx. 30% smaller than Nonpareil. The tree blooms approximately 8 d after Nonpareil and harvests approx. 18 d after Nonpareil. The kernels are symmetrical, medium in size and shallower than a typical Nonpareil shape making it desirable for the panning (Jordan-type sugar coated) almond. The kernel also has a slight but pleasant amaretto flavor, but this can vary year-to-year. Average kernel length/width/thickness is 2.3/1.2/0.8 cm. Ave. kernel weight is 0.9 g; kernel/kernel + shell crackout is 0.0.44. Shells are attractive and well-sealed, but we will still see some NOW damage (I suspect the amaretto flavor (benzaldehyde) is an attractant to NOW at these low levels). Prunus webbii and Sonora are in its lineage.